Trypsin enzyme

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Introduction to enzymes.

Enzymes are proteins found in nature that drive chemical reactions and break down or synthesize complex structures. They are biological catalysts accelerating chemical reactions. The molecules upon which enzymes may act are called substrates, and the enzyme converts the substrates into different molecules known as products. Almost all metabolic processes in the cell need enzyme catalysis in order to occur at rates fast enough to sustain life.

Enzymes are catalysts of a biological origin that are used for many processes across a broad spectrum of industries. Enzymes provide green solutions replacing chemicals in many industrial processes.

There are almost 4000 known enzymes, 200 of which are produced commercially and around 20 enzymes that are produced on an industrial scale. Food, beverages, detergents, textiles and the pharmaceutical industry are some of the major markets for industrial enzymes.

History of enzymes.

The use of food biotechnology dates back to thousands of years ago (6,000 B.C) to the time of the Sumerians and Babylonians. They then used yeast to make fermented beverages such as beer, baking bread, and cheese and wine, The use of plant enzymes such as malts were used millennia ago even before the actual understanding of enzymes.

in the late 1900th century **Christian Hansen** found the use of rennet (a mixture of Chymosin and Pepsin) in production of cheese and bacterial amylases started at Genencor. Rennet is an example of a natural enzyme mixture from the stomach of calves or other domestic animals that has been used in cheese making for centuries. Pectinases were used for juice clarification in the 1930s, during World War II, Invertase was also used for the production of invert sugar syrup in a process that pioneered the use of immobilized enzymes in the sugar industry. The large-scale application of enzymes only became really established in the 1960s, when the traditional acid hydrolysis of starch was replaced by an approach based in the use of amylases and amyloglucosidases (glucoamylases).

From then on, the trend for the design and implementation of processes and production of goods anchored in the use of enzymes has steadily increased. Enzymes extracted from edible plants and

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the tissues of food animals, and those produced by microorganisms (bacteria, yeasts, and fungi), have been used for centuries in process of food manufacturing.

In the twentieth century, enzymes began to be isolated from living cells, leading to a large-scale commercial production and with wider application in the food industry. Microorganisms being the most important source of commercial enzymes today. Enzyme manufacturers have optimized microorganisms for the production of enzymes through natural selection.

Enzyme technology has greatly benefited from the development of rDNA technologies, making production cheaper, efficient and feasible for manufacturers and thus made it possible to create high-value, application specific enzymes.

The global industrial enzymes market was valued at 4.2 Billion USD in 2014 and is expected to grow at 7% CAGR to potentially reach an overall value of 6.2 Billion USD by 2022. Novozymes A/S from Denmark, has the highest market share of an estimated 48% of the industrial enzymes market (Novozymes, 2019). DSM and DuPont are the main competitors in the market as well as several relatively small producers.

Some enzymes such as trypsin and carboxypeptidases can be used in the manufacturing of biopharmaceuticals and the demand for these enzymes will grow along with the growth in the biopharmaceutical industry.

Regulatory aspects of commercialization of enzymes

The regulation of enzymes internationally is quite varied between countries, with a specific country requiring either a full approval process, a notification of enzyme or no requirement thereof. Pre-market approval may depend on whether an enzyme is classified as processing aid or a food additive, though the point of consideration regardless of classification is that the safety of the enzyme must be assured. Challenges to regulators and industry arise from unresolved issues and from lack of harmonization of both legislation and safety evaluation**.**

As of April 2014, the major industry association AMFEP (Association of manufacturers and formulators of enzyme products) lists about 247 enzymes manufactured for the use in food industry. As enzymes are often used to replace steps in food processing encompassing harsh chemical or physical conditions they are perceived to be in line with both sustainable industrial production and careful processing of food in order to maintain nutritionally important ingredients such as vitamins, etc.

Enzymes have never been a focal issue either for regulators, consumer groups or the general public. In the beginning of 1990s this started to change when consumer and environmental groups were alarmed by increasing use of genetically modified microorganism for enzyme production.

In food legislation on food enzymes there has been a distinction on the basis of food additives and processing aids. Food additives are used for technological purposes and the substances are normally not consumed as food in itself or as a characteristic ingredient with nutritive value. Processing aids do not have any technological effect on finished food product. In Canada, USA and

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Japan for instance all food enzymes are regulated as food additives. In Australia food enzymes are considered as processing aids.

The joint FAO/WHO Expert committee on Food Additives (JECFA), has laid voluntary safety reviews on food enzymes since 1971 and does not differentiate on the basis of these above categories. In the EU food legislation, the food enzymes are considered as processing aids. Though this differentiation is not followed by the European commission's own scientific committee SCF (Scientific Committee on Food) which is responsible for evaluation of food additives and its toxicity.

US approval procedure of food enzymes.

Enzyme preparations can be regulated as secondary direct food additives under Title 21 of the Code of Federal Regulations, Part 173 (21 CFR 173). The regulatory status of food additives, including secondary food additives, is established through the petition process. According to Section 409(b)(1) of the Federal Food, Drug, and Cosmetic Act (the Act) [21 U.S.C. 348(b)(1)], anyone may file a petition proposing the issuance of a regulation. Section 409(b)(2) of the Act [21 U.S.C. 348(b)(2)] prescribes the statutory requirements for food additive petitions.

Section 201(s) of the Act [21 U.S.C. 321(s)] exempts the use(s) of a substance that is generally recognized as safe (GRAS) from the definition of a food additive. A substance can be determined to be GRAS under the intended conditions of use if there is evidence of its safety (the "technical element" of the GRAS standard) and a basis to conclude that this evidence is generally known and accepted by qualified experts.

Trypsin

Trypsin is a well-known enzyme consisting of a polypeptide chain of 223 amino acids after its proenzyme (trypsinogen), which is produced in the mammalian pancreas, is activated in the lumen of the intestine. Trypsin is a serine protease, therefore it hydrolyzes peptide bonds mainly at the carboxyl part of either lysine or arginine, except when either is followed by a proline. Trypsin extracted from animal sources usually contains chymotrypsin, which is another protease. When pure tryptic activity is desired, Trypsin is treated with Tosyl phenylalanyl chloromethyl ketone (TPCK). This may also add to the cost of using trypsin.

Trypsin has a broad range of applications such as leather processing, biotechnological processing, medicine, and food processing. It is widely used in biopharmaceutical manufacturing, especially for insulin and viral vaccines. The main functions of trypsin in the manufacturing of biopharmaceutical manufacturing are: 1) To detach adherent cells from each other and from the cell culture dish in order to passage them in cell cultures, 2) enzymatic conversion of insulin precursors into insulin and finally, 3) in influenza vaccine manufacturing where trypsin is utilized, in order to achieve higher yields of viruses from the cell cultures. Furthermore, another growing application, where trypsin has potential is in the field of regenerative medicine using cell culture technology. Here trypsin serves as a processing aid for the isolation of stem cells.

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Traditionally, the extraction of trypsin was mainly accomplished through animal sources, usually porcine or bovine. However, the increased awareness for potential viral contamination and the complex extraction process led to emergence of other means of enzyme extraction. Recombinant DNA technology made it possible to derive trypsin from non-animal sources, this trypsin is called "recombinant trypsin". Commonly used host organisms such as *E. coli*, *Pichia pastoris* or corn are able to synthesize trypsin, after they have been inserted with the mammalian gene fragment that codes for trypsin.

Trypsin applications include:

- *Process manufacturing applications in pharma such as insulin production and production of vaccines.*
- *Active pharmaceutical ingredient against conditions such as osteoarthritis and ulcers and wounds.*
- *Digestive aid*
- *Research applications such as in tissue cultures.*
- *Cosmetics*
- *Contact-lens cleaning solutions*

One way of analysing a trypsin business case is to use the SWOT analysis.

SWOT

Strengths may relate to the organisation and the trypsin product itself. Strength considerations in relation to the product may include:

Sustainability perspectives

The protease is very gentle on cells and offers high cell viability which is useful in all applications requiring cell culture technology

Recombinant trypsin may qualify for an animal origin free certification required for manufacturing in the biopharmaceutical industry.

Weaknesses may also relate to the organisation and the trypsin product itself. Weaknesses considerations in relation to the product may include:

Mixed availability of validation studies

Trypsin is a well-known enzyme with limited IPR protection options

Opportunities may include

The recombinant trypsin versions address the growing concern about animal-based viruses due to incidents in the past where the presence of adventitious agents originating from an animal source in a commercially available product, such as the presence of Porcine Circovirus (PCV) in Merck's

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rotavirus vaccine. A risk assessment here concluded that trypsin was the most likely source of contamination.

The rising trend towards “green chemistry” which is the use of sustainable chemical solutions benefits enzyme producers making substituting enzymes available, as companies want to reduce their environmental impact due to growing pressures from consumers and governing bodies.

As the egg-based production in vaccines is replaced in the manufacturing of viral vaccines with cell cultures, the market for trypsin will grow as well.

Advancements in stem cell therapy and its commercialization will increase the utility of trypsin and thereby grow the overall size of the market.

The growth in the size of the insulin market, along with the growth of its analogues will grow the market for trypsin.

Regulations in the animal health vaccines are less stringent than that in human health, thus it would be easier and less expensive to supply animal health vaccine manufacturers.

Threats may include:

Many competitors producing recombinant trypsin or animal derived trypsin

Innovations replacing trypsin such as the development of new cell dissociation enzymes or production systems that does not require trypsin.

Another way of analysing a trypsin business case is to use Porter´s Five Forces analysis.

Porter’s Five Forces:

Competitive rivalry: The abundance of similar products in the market is relatively high and there is little differentiation among the available products.

Threat of new entry: Even though the technology is not protected by means of patents anymore, the barrier of entry is high due to the volumes required in order to make the pricing competitive. The market is also very crowded with a small overall market size making it unprofitable for new entrants.

Threat of substitution: There are several products available in the market for recombinant trypsin. However, once a supplier has been chosen, buyers face high switching costs, therefore substitution is very unlikely.

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Buyer Power: There is an overall low degree of product differentiation and many options available, which gives buyers some negotiating power. The buyers face a very high cost of switching enzyme products, making them reliant on their enzyme suppliers in this industry.

Supplier power: Suppliers have generally lower power in the enzyme market.

Several factors can strongly influence the adoption of an enzyme in the manufacturing of biopharmaceuticals.

1. Cost: Cost of an enzyme is a major factor influencing the adoption of enzymes in the biopharmaceutical industry. Manufacturers of biopharmaceuticals see the cost of enzymes as a challenge as an increase in the expense of enzymes would reduce profit margins. Enzymes are costly to produce and enzymes that are not widely used, are more expensive because they are not produced at a large scale. As more companies adopt the use of enzymes, the cheaper the enzymes will become in the future due to growth in competition and economies of scale, over time. Costs will also reduce with process innovations in the manufacturing of enzymes and host expression systems.
2. Regulations: Regulations are a key factor that can both enhance or reduce the adoption of an enzyme for manufacturing processes.
   The regulatory authorities enforce regulations and expect companies to comply with certain regulations, such as cGMP quality compliance, in the case of FDA. It should be highlighted that cGMP compliance is a minimum requirement for manufacturing of pharmaceuticals and many of the companies already surpass the quality standards by far (FDA, 2018). Regulatory bodies such as the EMA recommend the manufacturing of enzymes such as trypsin according to GMP, ISO or HACCP-compatible quality systems (EMA, 2014). Regulations can enhance product adoption by necessitating newer protocols that ensure safety and quality compliance management systems, such as recommendations by authorities on the usage of animal origin free products.
3. Scientific evidence: In order to adopt an enzyme, manufacturers have to be convinced by means of scientific evidence about the benefits of the innovation through validations studies and academic papers as shown by the results.
4. Supplier assets: Manufacturers prefer suppliers with a history of consistency and stability to ensure the supply chain isn't broken by shutting down of the production facility of the supplier. This makes assets such as a consistent supply chain and brand reputation a crucial aspect in the decision to adopt a enzyme product in manufacturing of biopharmaceuticals.
5. Safety: Safety, apart from what is mandated by regulations, is an important internal requirement for the biopharmaceutical companies.
6. Relative advantage: The relative advantage of product characteristics such as improved gentleness or activity which would increase product yields or efficiency of the process are desired. Other aspects are improved stability, which can reduce long term costs for manufacturers due to ease of storage and increased purity, which can in turn reduce the costs in downstream processing, are all considered by manufacturers.
7. Development stage: The findings show that the likelihood of a new enzyme being adopted in the manufacturing process of a biopharmaceutical product is greater in the early development stages, due to the ease in switching during the early development stages. The product and processes in manufacturing of biopharmaceuticals are closely linked. The

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regulations in manufacturing are also very stringent, which makes it a very expensive and time-consuming process for a manufacturer to switch to a new product, despite even its relative advantages once a product gets past the early development phase. Thus, the adoption of the enzyme would depend on the development phase of the target biopharmaceutical.

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